

Claims:

1. A method for separating an ampholytic component by electrophoresis, the method comprising:

5 placing a sample containing an ampholytic component having a pI value in an electrophoresis separation system comprising an anolyte having a pH and a catholyte having a pH, the catholyte pH being higher than the anolyte pH, one or more ion-permeable barriers disposed between the anolyte and catholyte wherein at least one of the barriers is an isoelectric barrier having a pI value which is higher than the anolyte pH and lower than the catholyte pH;

10 providing an isoelectric buffer having a pI value higher than the pH of the anolyte and lower than the pH of the catholyte and different from the pI value of the ampholytic sample component and different from the pI value of an ion-permeable isoelectric barrier; and

15 exposing the sample to an electric potential so as to trap the ampholytic sample component in a non-isoelectric state in the presence of the isoelectric buffer in the electrophoresis system.

2. The method according to claim 1 wherein the electrophoretic separation system comprises an anode compartment containing an anode and adapted to receive the anolyte, a cathode compartment containing a cathode and adapted to receive the catholyte, and one ion-permeable barrier in the form of an ion-permeable isoelectric barrier having a pI value disposed between the anode compartment and the cathode compartment.
3. The method according to claim 2 wherein the electrophoretic separation system further comprises first and second ion-permeable barriers disposed between the anode compartment and the cathode compartment forming a first separation compartment between the anode compartment and cathode compartment.
4. The method according to claim 3 wherein the first and second ion-permeable barriers are ion-permeable isoelectric barriers each having a defined but different pI.
5. The method according to claim 3 or 4 wherein the sample is provided to at least one of the sample compartment, anode compartment or the cathode compartment.
6. The method according to claim 5 wherein the sample is provided to the sample compartment and the ampholytic component is separated in a non-isoelectric state in the sample compartment.

7. The method according to claim 5 wherein the ampholytic component is obtained in a non-isoelectric state in the sample compartment or the cathode compartment or the anode compartment.
- 5 8. The method according to claim 3 or 4 wherein the sample contains two or more ampholytic components which are obtained in non-isoelectric states in the sample compartment or the cathode compartment or the anode compartment or two or more of the sample compartment, the anode compartment or the cathode compartment.
- 10 9. The method according to claim 3 wherein the electrophoretic separation system further comprises another ion-permeable barrier disposed between the anode compartment and the cathode compartment forming a second separation compartment adjacent the first separation compartment, the system having first ion-permeable isoelectric barrier having a first pI value and a second ion-permeable isoelectric barrier having a second pI value different from the first pI value of the first ion-permeable isoelectric barrier.
- 15 10. The method according to claim 9 wherein the ion-permeable barriers are all isoelectric barriers each having a defined but different pI.
11. The method according to claim 9 or 10 wherein the sample is provided to the first sample compartment, the second sample compartment, the anode compartment or the cathode compartment, or both the first and second sample compartments.
- 20 12. The method according to claim 11 wherein the ampholytic component is obtained in a non-isoelectric state in the first sample compartment or in the second sample compartment, or in the anode compartment, or the cathode compartment.
- 25 13. The method according to claim 9 or 10 wherein the sample contains two or more ampholytic components which are obtained in a non-isoelectric state in the first sample compartment or in the second sample compartment, or in each of the first sample compartment and second sample compartment.
- 30 14. The method according to claim 2 wherein the electrophoretic separation system further comprises a plurality of ion-permeable barriers disposed between the anode compartment and the cathode compartment forming a plurality of separation compartments disposed between the anode and cathode compartments, wherein two or more ion-permeable barriers are ion-permeable isoelectric barriers each having a different pI value.
15. The method according to claim 14 wherein three or more of the ion-permeable barriers are ion-permeable isoelectric barriers each having a different pI value.

16. The method according to claim 14 wherein all the ion-permeable barriers are ion-permeable isoelectric barriers each having a different pI value.
17. The method according to any one of claims 9 to 16 wherein a first isoelectric buffer and a second isoelectric buffer each having a different pI value are provided to at least two of the sample compartments or the anode compartment or the cathode compartment.
18. The method according to any one of claims 1 to 17 wherein the ion-permeable barriers are selected from the group consisting of immiscible liquids, porous solids, non-ionic membranes, membranes, isoelectric membranes, hydrogel membranes, gels, and hydrogels.
19. The method according to 18 wherein the hydrogel membranes are polyether sulfone-based, poly(vinyl) alcohol-based or polyacrylamide-based.
20. The method according to claim 18 wherein the non-ionic membranes are supported membranes selected from the group consisting of cross-linked polyacrylamide and poly(vinyl) alcohol supported on glass fiber, filter paper, polymeric mesh, and paper.
21. The method according to claim 18 wherein the ion-permeable barriers are porous frits selected from the group consisting of glass frits, ceramic frits, and polymeric frits.
22. The method according to any one of claims 18 to 21 wherein the ion-permeable barriers substantially restrict the passage of molecules having a size or hydrated ion radius greater than a predetermined size.
23. The method according to any one of claims 1 to 17 wherein the ion-permeable isoelectric barriers are selected from the group consisting of isoelectric porous solids, isoelectric membranes, and isoelectric gels.
24. The method according to claim 23 wherein the ion-permeable isoelectric barriers are isoelectric membranes.
25. The method according to any one of claims 1 to 24 wherein there is substantially no convective mixing between the compartments.
26. The method according to any one of claims 1 to 25 wherein the isoelectric buffer is provided to at least one of a sample compartment, the anode compartment and the cathode compartment
27. The method according to claim 26 wherein the pI value of the isoelectric buffer differs by at least 0.001 pH units from the pI value of the ampholytic sample component.

28. The method according to any one of claims 1 to 27 wherein the absolute value of the difference between the pI value and the pKa value closest to the pI value of the isoelectric buffer is less than about 1.5.
29. The method according to any one of claims 1 to 28 wherein the isoelectric buffer is  
5 selected from the group consisting of iminodiacetic acid, N-methylimino diacetic acid, aspartic acid, glutamic acid, glycyl-aspartic acid, m-aminobenzoic acid, histidyl-glycine, histidyl-histidine, histidine, 1,2-diaminopropionic acid, ornithine, lysine, lysil-lysine, and arginine.
30. The method according to claim 29 wherein the isoelectric buffer is glutamic acid or  
10 lysine.
31. The method according claim 17, wherein the first isoelectric buffer is glutamic acid and the second isoelectric buffer is lysine.
32. The method according to any one of claims 1 to 31 further comprising providing a non-ionic solubilizing agent.
- 15 33. The method according to claim 32 wherein the solubilizing agent is a non-ionic detergent.
34. The method according to any one of claims 1 to 33 wherein the ampholytic sample component is selected from the group consisting of proteins, polypeptides, oligopeptides, amino phenols, amino phosphonic acids, and amino acids.
- 20 35. The method according to claim 35, wherein the ampholytic sample component is a protein.